AD-A051 580 ARMY MEDICAL RESEARCH INST OF INFECTIOUS DISEASES FR--ETC F/G 6/15
EVALUATION OF A NUCLEASE-RESISTANT DERIVATIVE OF POLY(I) POLY(C--ETC(U)
MAR 78 W B BAZE, E LVOVSKY, G A HIGBEE

UNCLASSIFIED NL

| OF |
AD
A051580

END
DATE
FILMED
4 -78
DDC

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
(6)		(9)

4. TITLE (and Subtitle)

Evaluation of a Nuclease-Resistant Derivative of Poly(I)·Poly(C) [Poly(ICLC)] as a Radioprotective Agent 1, 2.

5. TYPE OF REPORT & PERIOD COVERED

Interim rept.

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)

(10) Wallace B. Baze, Eduard Lvovsky, Glen A. Higbee, Hilton B. Levy [redacted] Duane E. Hilmas

8. CONTRACT OR GRANT NUMBER(s)

9. PERFORMING ORGANIZATION NAME AND ADDRESS

U.S. Army Medical Research Institute of Infectious Diseases SGRD-UIA-A
Fort Detrick, Frederick, Maryland 21701

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

(16) 62776A (17) 3M762776A841 00 008

11. CONTROLLING OFFICE NAME AND ADDRESS

(11)

12. REPORT DATE

1 Mar [redacted] 78

13. NUMBER OF PAGES

24

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

15. SECURITY CLASS. (of this report)

UNCLASSIFIED (12) 26p.

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

DDC RECEIVED MAR 22 1978 D

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Reprints bearing assigned AD number will be forwarded upon receipt.
To be submitted for publication in Radiation Research.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Polyriboinosinic acid·polyribocytidylic acid complex with poly-l-lysine
Interferon
Radioprotection

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Intramuscular injection of a stabilized polyriboinosinic acid·polyribocytidylic acid complex, composed of the two acids, poly-l-lysine and carboxymethylcellulose [poly(ICLC)], was radioprotective in mice when given in a single dose 48, or 72, or in multiple doses 24 and 48, or 24, 48 and 72 hr before total-body ^{60}Co irradiation. At dosages of 0.1-3.0 mg/kg, $LD_{50/30}$ values were increased significantly ($P < 0.05$), from 86 to 157 rads with a maximum dose reduction factor of 1.25. Survival rates and weighted mean survival times were also significantly increased. Increased circulating levels of serum interferon induced by poly(ICLC) (cont'd)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

405 039

were not required at the time of irradiation for radioprotection. Poly(ICLC) was found to have a slight stimulatory effect on the numbers of peripheral blood elements in irradiated mice.

Evaluation of a Nuclease-Resistant Derivative of Poly(I)·Poly(C) [Poly(ICLC)] as a Radioprotective Agent[1,2]

WALLACE B. BAZE,* EDUARD LVOVSKY, GLEN A. HIGBEE, HILTON B. LEVY, AND DUANE E. HILMAS†

U. S. Army Medical Research Institute of Infectious Diseases
Fort Detrick, Frederick, Maryland 21701 and
National Institute of Allergy and Infectious Diseases,
Bethesda, Maryland 20014

Number of copies submitted: 3

Manuscript pages: 21

Figures: 3

Tables: 1

*Present address: CPT Wallace B. Baze, VC,
Institute of Surgical Research,
Fort Sam Houston, Texas 78234

†Present address: LTC Duane E. Hilmas, VC,
Chief, Office for Wholesomeness of Irradiated Foods,
U.S. Army Medical Research and Development Command,
Washington, D.C. 20314

Running head: EVALUATION OF POLY(ICLC) AS A RADIOPROTECTANT.

Send proofs to: c/o Mrs. Regina Staley

U. S. Army Medical Research Institute of

Infectious Diseases

Fort Detrick, Maryland 21701

ABSTRACT

Intramuscular injection of a stabilized polyriboinosinic acid.polyribocytidylic acid complex, composed of the two acids, poly-l-lysine and carboxymethylcellulose [poly(ICLC)], was radioprotective in mice when given in a single dose 48, or 72, or in multiple doses 24 and 48, or 24, 48 and 72 hr before total-body ^{60}Co irradiation. At dosages of 0.1-3.0 mg/kg, $LD_{50/30}$ values were increased significantly ($P < 0.05$), from 86 to 157 rads with a maximum dose reduction factor of 1.25. Survival rates and weighted mean survival times were also significantly increased. Increased circulating levels of serum interferon induced by poly(ICLC) were not required at the time of irradiation for radioprotection. Poly(ICLC) was found to have a slight stimulatory effect on the numbers of peripheral blood elements in irradiated mice.

Key Words:

(1) Polyriboinosinic acid·polyribocytidylic acid complex with poly-l-lysine (2) interferon, and (3) radioprotection

INTRODUCTION

The synthetic double-stranded ribonucleic acid, polyriboinosinic acid·polyribocytidylic acid (poly(I)·poly(C)) is an interferon inducer (1), has antiviral (2-4) and antitumor (5-7) properties, and has been shown to be radioprotective in rodents (8, 9, O. V. Semina, A. M. Poverenny and A. G. Konoplyannikov, personal communication). The observation that the radioprotective action of poly(I)·poly(C), as well as certain endotoxins (10) and chemicals (11, 12), may be associated with their ability to induce interferon, has also stimulated interest in the possible radioprotective action of interferon itself (13).

Poly(I)·poly(C), although an effective interferon inducer in rodents, induces little or no interferon in nonhuman primates and man due to a hydrolytic nuclease in primate sera (14). However, poly(I)·poly(C), complexed with poly-l-lysine and carboxymethylcellulose, designated poly(ICLC), is 4-10 times more resistant to hydrolysis than the parent compound (15). Poly(ICLC) induces moderate to high levels of serum interferon in rodents (16), nonhuman primates (17), and man (H. B. Levy and A. Levine, personal communication), and in addition has antiviral (18-21) and adjuvant (22, D. G. Harrington, personal communication) properties in rodents and monkeys. This compound had also been used in some cancer therapy protocols in man as an experimental chemotherapeutic agent (H. B. Levy and A. Levine, personal communications).

This paper describes radioprotection achieved in total-body irradiated mice pretreated with poly(ICLC), and shows that high levels of circulating interferon are not required for radioprotection.

MATERIALS AND METHODS

Drug

Poly(ICLC), 2 mg of poly(I)·poly(C)/ml, was prepared as previously described (14). For injection into mice, poly(ICLC) was appropriately diluted with pyrogen-free 0.85% NaCl (saline) so that final injection volumes were 0.1 ml.

Animals and Animal Inoculations

Male Swiss-Webster mice (Dub:ICR), 30-40 days old, weighing approximately 30 g, were used in all experiments. Mice were housed 4 or 16 per pan and given fresh water and Wayne Lab-Blox *ad libitum*.

Groups of 8 to 20 mice selected at random were used in all experiments. All injections were given intramuscularly (IM) in a hind leg. Multiple injections were alternated between both hind legs. Poly(ICLC) was used in dosages of 0.1, 0.3, and 1.0 and 3.0 mg/kg. Mice inoculated with saline served as controls.

Irradiation

Total-body irradiation exposures were accomplished by using a bilateral 40,000 Ci ^{60}Co source as previously described (23).

Mice were exposed individually in a compartmentalized polyethylene exposure chamber, rotated at approximately 1 rpm. Dose rate was 50 rads/min.

For radioprotection studies mice were irradiated in 100 rad increments from 400 to 1000 rads. Mice were given poly(ICLC) either as a single dose 8, 24, 48 or 72 hr before irradiation, or in multiple doses 24 and 48 or 24, 48 and 72 hr before irradiation.

Interferon

Serum interferon (reference units per milliliter) was assayed by reduction of cytopathic effects (CPE) induced by vesicular stomatitis virus in tissue culture of mouse L-929 cells. A control group of mice was inoculated IM with saline and bled 24 hr later to serve as a negative control in the interferon assay.

Hematology

Selected peripheral blood elements were studied in three groups of mice as follows: (1) those given poly(ICLC) in two doses, 24 and 48 hr before irradiation (600 rads), (2) those given two doses of drug alone, and (3) irradiation controls given saline in lieu of poly(ICLC).

Two preexperimental blood samples were collected in ethylenediaminetetraacetic acid from the orbital sinus of each mouse to determine base-line mean white blood cell (WBC), neutrophil, lymphocyte, platelet, and packed cell volume (PCV) values. Mice from each experimental group were bled on days 2, 7, 14 and 29 after the second drug dose. Each postinoculation blood cell count was divided by its corresponding mean base-line value and multiplied by 100 to obtain a normalized value. A group mean normalized value of 100% indicates no deviation from base line, whereas a value of 24% indicates an approximately 76% decrease from the preexperimental base-line value.

Statistics

Postirradiation survivors were counted daily for at least 30 days after irradiation. Percentage survivors and dose reduction factor (DRF) were calculated as previously described (24). Significance of differences in percentage survivors compared to controls were determined by a chi-square test with Yates' correction. A weighted mean survival time (WMST) was

calculated (25). Since death patterns for mice from group to group did not always follow a normal distribution, the Kolmogorov-Smirnov two-sample test (26) was used to compare the lethality distribution pattern of mice in poly(ICLC)-treated vs. saline-treated groups. The radiation dose that killed 50% of the mice in 30 days ($LD_{50/30}$) was calculated by probit analysis (27).

RESULTS

Survival rates were increased in mice protected with poly(ICLC) and irradiated at doses from 400 to 900 rads when compared to survival rates of saline-treated, irradiated control mice (Fig. 1). Survival rates were increased significantly ($P < 0.05$) in the 600, 700 and 800 rad dose groups treated with poly(ICLC) compared to saline controls at the same radiation dose (Fig. 1). Radiation $LD_{50/30}$ values of poly(ICLC)-treated mice were increased with a calculated DRF in the range of 1.14 to 1.25 (Fig. 1). For example, the $LD_{50/30}$ values for mice given a single dose of drug (0.3 mg/kg) 48 or 72 hr before irradiation were 764 and 765 rads, respectively, compared to 608 rads in control mice (Fig. 1). The DRF for both groups of mice was 1.25. Survival rates of mice given poly(ICLC) 8 or 24 hr before irradiation were increased when compared to those of irradiated controls, but not as consistently nor as pronounced as earlier treatment times.

The WMST was increased significantly ($P < 0.05$) for all poly(ICLC)-treated groups compared to WMST for saline-treated, irradiated controls (Fig. 2). Mice given a single dose of drug (0.3 mg/kg) 48 or 72 hr before 700 rads had WMST of 26.3 and 29.3 days, respectively, compared to 16.8 days for control mice (Fig. 2). The WMST for mice given two or three daily doses of poly(ICLC) before irradiation was 7.9 to 10.1 days longer than controls (Fig. 2). Percentage survivors were increased in the single and multiple poly(ICLC) dose groups given 700 rads compared to control mice given 700 rads (Fig. 2).

The temporal relationship of poly(ICLC)-induced serum interferon titers to time of irradiation for maximum radioprotection are shown in figure 3. Following a single dose of drug, serum interferon titers were maximum in 24 hr, but barely detectable at 48 and 72 hr, the time of irradiation that yielded maximum radioprotection (Fig. 3). Following two doses of drug, 24 and 48 hr before irradiation, or three doses of drug, 24, 48 and 72 hr before irradiation, serum interferon titers were again maximum 24 hr after the first dose; however, with additional doses, hyporesponsiveness ensued (Fig. 3). The greatest increase in percentage survivors occurred when serum interferon titers were low at the time of irradiation (Fig. 3). The serum interferon response of mice increased as the dose of poly(ICLC) increased (Fig. 3).

Following two daily doses of poly(ICLC), all hematologic variables measured were increased slightly in mice through the 29-day experimental period (Table 1). WBC, neutrophil, lymphocyte, platelet and PCV values for mice pretreated with either poly(ICLC) or saline decreased subsequent to irradiation (600 rad) as expected (Table I). However, all variables were increased slightly in mice given poly(ICLC) prior to irradiation when compared to similar values for saline-treated, irradiated controls (Table I). For both irradiated groups, WBC and neutrophil counts were decreased from base-line values from day 2 through 14 with recovery to normal levels by day 29. Lymphocyte counts were decreased from day 2 through 14 with incomplete recovery by day 29 in both irradiated groups. Platelet counts were increased on day 2, then decreased before returning to base-line values on day 29. PCV values were relatively unaffected by irradiation or phlebotomy (Table I).

DISCUSSION

This report shows that poly(I)·poly(C) stabilized with poly-l-lysine and carboxymethylcellulose [poly(ICLC)] is a radioprotectant. Poly(ICLC) given to mice in single or multiple doses by the IM route before total-body radiation exposure increases percentage survivors when irradiated in the dose range of 400 to 900 rads. Maximum radioprotective effect occurred when poly(ICLC) was given at 0.1-3.0 mg/kg in a single dose 48 or 72 hr before irradiation or in multiple doses 24 and 48, or 24, 48 and 72 hr before irradiation. At these treatment times, radiation $LD_{50/30}$ increased 86 to 157 rads, with a maximum DRF of 1.25. The WMST of drug-treated, irradiated mice was increased over that of counterpart irradiated controls. However, drug dosages above 3.0 mg/kg failed to cause further increases in these variables over that of control mice even though interferon titers continued to increase with increased drug doses (W. B. Baze and D. E. Hilmas, unpublished data).

Survival rate, $LD_{50/30}$, and WMST were not increased consistently when poly(ICLC) was given in single dose 8 or 24 hr before irradiation. In addition, other studies show that poly(ICLC) given to mice 30 min before irradiation as well as 30 min and 1 to 7 days after irradiation failed to alter any of these variables significantly (W. B. Baze and D. E. Hilmas, unpublished data).

These findings are in agreement with observations of others who have shown that poly(I)·poly(C) increases survival rates of mice when given 48 hr before total-body irradiation (8, 9, O. V. Semina, et al., personal communication). Likewise, crude homologous interferon administered to mice 24 hr before total-body irradiation causes a moderate increase in WMST after 600 rads (28). Heterologous interferon

(rabbit) is less radioprotective in mice (28). In addition, partly purified interferon administered 2 days before irradiation has been shown to increase endogenous spleen colonies and survival rates of irradiated mice (29).

To what are increased survival rates attributed, and are high circulating interferon titers required for radioprotection? Radioprotection of mice with nonstabilized poly(I)·poly(C) has been attributed to an increase in the fraction of hematopoietic stem cells in the irradiated host, mediated through induced interferon (8, O. V. Semina et al., personal communication). It was found that poly(I)·poly(C)-treated mice produce 12 times more spleen colonies by day 10 postirradiation in contrast to control mice (E. Szolgay and M. Talas, personal communication). These authors suggested that maximum serum interferon titers correlated with increased spleen colony formation and resulted in radioprotection.

In contrast, our data show that peak serum interferon titers in comparison to time of irradiation for maximum radioprotection do not coincide. Irradiation of poly(ICLC)-treated mice at the time of peak serum interferon response failed to show any increase in survival rates compared to survival rates of mice irradiated when circulating interferon titers are low or not detectable. It is apparent that high circulating levels of interferon are not required at the time of irradiation for radioprotection.

Other studies (30) show that poly(I)·poly(C) also increases the in vivo hematopoietic colony-stimulating activity of mouse serum; this activity was inversely related to poly(I)·poly(C)-induced serum interferon titers. That is, serum interferon titers peaked at 6 to 8 hr after a single dose of poly(I)·poly(C) and were negligible

at 48 hr when colony-stimulating activity was 3 to 4 times normal. Hyporesponsiveness to interferon induction ensued following additional doses of poly(I)·poly(C); however, colony-stimulating activity was 1 to 3 times normal levels for 48 hr after each additional dose of drug (30). Serum inteferon responses following single and multiple doses of poly(ICLC) are consistent with the poly(I)·poly(C) data (30). It is likely that poly(ICLC) also increases serum hematopoietic colony-stimulating activity when serum interferon titers are low. A slight increase in the number of peripheral blood elements was noted in both nonirradiated and irradiated mice given poly(ICLC). These increases in peripheral blood elements for mice given poly(ICLC) before irradiation are circumstantial indicators of increased hematopoietic activity.

The inverse relationship between serum hematopoietic colony-stimulating activity and poly(I)·poly(C)-induced serum interferon titers has been explained by the presence of a colony inhibitor, related to interferon, detected in sera of mice (30, 31). The amount of colony-inhibiting activity in whole mouse serum is directly proportional to the amount of serum interferon; colony inhibitor and interferon were also found to have similar physical-chemical properties, such as molecular size, pH, species specificity, and trypsin (31).

An inhibitory effect by interferon on hematopoietic tissues has been suggested by others (29, 32, O. V. Semina et al., personal communication). This inhibitory effect may be followed by stimulation of these same tissues by other factors produced in association with interferon (13). That interferon may be inhibitory to hematopoietic tissues, while factors produced in association with interferon may be stimulatory, helps to explain radioprotection found with crude

preparations of interferon (28, 29) or with interferon-inducers such as poly(I)·poly(C) (8, 9, O. V. Semina et al., presonal communication) or poly(ICLC). Consistent with this explanation is the observation that highly purified interferon lacks any radioprotective effect (E. Lvovsky, unpublished data). Further, the fact that 10 mg/kg of poly(ICLC) induces very high levels of serum interferon in mice (W. B. Baze and D. E. Hilmas, unpublished data), yet fails to protect mice, supports this hypothesis. The possibility that biologically active compounds produced in association with interferon may aid or stimulate postirradiation recovery by the hematopoietic or other system needs further investigation. Also, the potential for poly(ICLC) to be used in combination as a chemotherapeutic and radioprotective agent in cancer therapy should be explored.

REFERENCES

1. A. K. FIELD, A. A. TYTELL, G. P. LAMPSON, and M. R. HILLEMAN, Inducers of interferon and host resistance. II. Multistranded synthetic polynucleotide complexes. Proc. Nat. Acad. Sci. U. S. A. 58, 1004-1010 (1967).

2. H. F. LINDH, H. L. LINDSAY, B. R. MAYBERRY, and M. FORBES, Polyinosinic-cytidylic acid complex (polyI:C) and viral infections in mice. Proc. Soc. Exp. Biol. Med. 132, 83-87 (1969).

3. G. D. MAYER and R. F. KRUEGER, Antiviral comparisons of statolon and polyinosinic-polycytidylic acid. In Antimicrobial Agents and Chemtherapy--1969 (G. L. Hobby, Ed.), pp. 182-186. American Society for Microbiology, Bethesda, MD, 1969.

4. M. M. NEMES, A. A. TYTELL, G. P. LAMPSON, A. K. FIELD, and M. R. HILLEMAN, Inducers of interferon and host resistance. VI. Antiviral efficacy of poly I:C in animal models. Proc. Soc. Exp. Biol. Med. 132, 776-783 (1969).

5. H. B. LEVY, R. ASOFSKY, F. RILEY, A. GARAPIN, H. CANTOR, and R. ADAMSON, The mechanism of the antitumor action of poly I: poly C. Ann. NY. Acad. Sci. 173, 640-648 (1970).

6. H. B. LEVY, Interferon and interferon inducers in the treatment of neoplastic diseases. In Cancer Chemotherapy II, Twenty-second Hahnemann Symposium (I. Brodsky and S. B. Kahn, Eds.), pp. 53-61. Grune and Stratton, New York, 1972.

7. M. LIEBERMAN, T. C. MERIGAN, and H. S. KAPLAN, Inhibition of radiogenic lymphoma development in mice by interferon. Proc. Soc. Exp. Biol. Med. 138, 575-578 (1971).

8. O. V. SEMINA, A. G. KONOPLYANNIKOV, and A. M. POVERENNY, Action of some biologically active preparations on cell population of stem hemopoiesis fraction and on the survival of irradiated mice. Comm. 2. Polyinosinic-polycytidylic acid. Radiobiologia 14, 686-689 (1974).

9. E. V. BINIASHEVSKY, and N. K. TERNOVOY, Effect of interferon on the unspecific radioresistance of white mice to roentgen irradiation. Vrach. Delo. (7), 138-141 (July 1975).

10. W. W. SMITH, Hemopoiesis-promoting effect of endotoxin in irradiated animals. In Bacterial Endotoxins (M. Landy and W. Braun, Eds.), pp. 205-210. Institute of Microbiology, Rutgers, The State University, New Brunswick, NJ (1964).

11. E. LVOVSKY, H. B. LEVY, D. G. DOHERTY, and S. BARON, Interferon induction by radioprotective mercaptoalkylamines and derived thiophosphates. Infect. Immun. 15, 191-196, (1977).

12. E. LVOVSKY, H. G. DUBUY, and H. B. LEVY, Interferon induction by histamine, serotonin and mexamine. Abstracts of the Annual Meeting--1977, p. 290. American Society for Microbiology, Bethesda, MD, 1977.

13. E. LVOVSKY, W. B. BAZE, D. E. HILMAS and H. B. LEVY, Radiation damage and the interferon system. Texas Rep. Biol. Med. 35, 388-393 (1977).

14. J. J. NORDLUND, S. M. WOLFF, and H. B. LEVY, Inhibition of biologic activity of poly I: poly C by human plasma. Proc. Soc. Exp. Biol. Med. 133, 439-444 (1970).

15. H. B. LEVY, G. BAER, S. BARON, C. E. BUCKLER, C. J. GIBBS, M. J. IADAROLA, W. T. LONDON, and J. RICE, A modified polyriboinosinic-polyribocytidylic acid complex that induces interferon in primates. J. Infect. Dis. 132, 434-439 (1975).

16. W. B. BAZE, E. LVOVSKY, and D. E. HILMAS, Evaluation of a nuclease-resistant derivative of poly(I):poly(C) as a radioprotective agent. Radiat. Res. 70, 623 (1977).

17. M. L. SAMMONS, E. L. STEPHEN, H. B. LEVY, S. BARON, and D. E. HILMAS, Interferon induction in cynomolgus and rhesus monkeys after repeated doses of a modified polyriboinosinic-polyribocytidylic acid complex. Antimicrob. Agents Chemother. 11, 80-83 (1977).

18. R. W. KUEHNE, W. L. PANNIER, and E. L. STEPHEN, Indirect mouse model for the evaluation of potential antiviral compounds: results with Venezuelan equine encephalomyelitis virus. Antimicrob. Agents Chemother. 11, 683-687 (1977).

19. E. L. STEPHEN, M. L. SAMMONS, W. L. PANNIER, S. BARON, R. O. SPERTZEL, and H. B. LEVY, Effect of a nuclease-resistant derivative of poly(I)·poly(C) on yellow fever in rhesus monkeys. J. Infect. Dis. 136, 122-126 (1977).

20. G. M. BAER, J. H. SHADDOCK, S. A. MOORE, P. A. YAGER, S. BARON, and H. B. LEVY, Successful prophylaxis against rabies in mice and rhesus monkeys: the interferon system and vaccine. J. Infect. Dis. 136, 286-292 (1977).

21. R. H. PURCELL, W. T. LONDON, V. J. McAULIFFE, A. E. PALMER, P. M. CAPLAN, J. L. GERIN, J. WAGNER, H. POPPER, E. LVOVSKY, D. C. WONG, and H. B. LEVY, Modification of chronic hepatitis-B virus infection in chimpanzees by administration of an interferon inducer. Lancet 2, 757-762 (1976).

22. W. E. HOUSTON, C. L. CRABBS, E. L. STEPHEN, and H. B. LEVY, Modified polyriboinosinic-polyribocytidylic acid, an immunological adjuvant. Infect. Immun. 14, 318-319 (1976).

23. R. E. CARTER and D. E. VERRILLI, AFRRI cobalt whole-body irradiator. AFRRI Technical Note TN 73-3, March 1973.

24. J. M. YUHAS, Biological factors affecting the radioprotective efficiency of S-2-[3-aminopropylamino]ethylphosphorothioic acid (WR2721). $LD_{50(30)}$ doses. Radiat. Res. 44, 621-628 (1970).

25. F. F. PINDAK, J. P. SCHMIDT, D. J. GIRON, and P. T. ALLEN, Interferon levels and resistance to viral infection associated with selected interferon inducers. Proc. Soc. Exp. Biol. Med. 138, 317-321 (1971).

26. S. SIEGEL, In Non-parametric Statistics for the Behavioral Sciences, pp. 127-136. McGraw Hill, New York 1956.

27. D. J. FINNEY, Probit Analysis. 3rd Edition. Cambridge University Press, Cambridge (1971).

28. A. G. KHAITOVICH, E. A. LVOVSKY, and P. N. KISELEV, Radioprotective action of exogenous interferon. Radiobiologia 14, 356-358 (1974).

29. O. V. SEMINA, YU. A. NEIFAKH, E. L. SABOLEVA, A. G. KONOPLYANNIKOV, and A. M. POVERENNY, Action of some biologically active preparations on a population of cells of stem haemopoietic fraction and on the survival of irradiated mice. Comm. 3. Interferon. Radiobiologia 16, 218-222 (1976).

30. T. A. McNEILL and M. KILLEN, The effect of synthetic double-stranded polyribonucleotides on haemopoietic colony-forming cells in vivo. Immunology 21, 751-759 (1971).

31. T. A. McNEILL and W. A. FLEMING, The relationship between serum interferon and an inhibitor of mouse haemopoietic colonies *in vitro*. *Immunology* 21, 761-766 (1971).

32. J.-C. CEROTTINI, K. T. BRUNNER, P. LINDAHL, and I. GRESSER, Inhibitory effect of interferon preparations and inducers on the multiplication of transplanted allogeneic spleen cells and syngeneic bone marrow cells. *Nature* (*New Biol.*) 242, 152-153 (1973).

FOOTNOTES

[1]In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

[2]The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

TABLE I

Effect of 0.3-3.0 mg/kg Poly(ICLC) and Irradiation Treatment on Peripheral Blood Elements of Mice (N = 10-20)

Blood element	Poly(ICLC)[a]	Poly(ICLC)[b]+ 600 rads	600 Rads[c]	% Change from base line by days			
				2	7	14	29
WBC	+	-	-	117	160	122	125
	-	+	-	36	17	20	91
	-	-	+	24	7	15	99
Neutrophils	+	-	-	136	242	155	110
	-	+	-	91	36	24	133
	-	-	+	63	13	14	209
Lymphocytes	+	-	-	114	134	121	138
	-	+	-	14	10	23	72
	-	-	+	12	6	19	66
Platelets	+	-	-	100	137	135	131
	-	+	-	125	52	37	102
	-	-	+	114	81	25	83
PCV	+	-	-	101	114	119	118
	-	+	-	106	102	97	122
	-	-	+	108	92	84	121

[a]Two doses of poly(ICLC) 24 hr apart.

[b]Two doses of poly(ICLC) 24 and 48 hr before irradiation.

[c]Two doses of saline 24 and 48 hr before irradiation.

LEGENDS FOR FIGURES

FIG. 1. Percentage survivors, $LD_{50/30}$ and dose reduction factor (DRF) for mice (n = 16-48) given poly(ICLC) at various times, (A) 48 hr, (B) 72 hr, (C) 24 and 48 hr, and (D) 24, 48 and 72 hr, before total-body irradiation. ▲, 3.0 mg/kg; ▼, 1.0 mg/kg; ■, 0.3 mg/kg; ◆, 0.1 mg/kg; ●, saline controls; *P < 0.05; (95% confidence interval).

FIG. 2. Kinetics of death, weighted mean survival time (WMST) and percentage survivors for mice (n = 12-48) given poly(ICLC) at various times, (A) 48 hr, (B) 72 hr, (C) 24 and 48 hr, and (D) 24, 48 and 72 hr, before total-body irradiation (700 rads). ▲, 3.0 mg/kg; ▼, 1.0 mg/kg; ■, 0.3 mg/kg; ◆, 0.1 mg/kg; ●, saline controls; *P < 0.05.

FIG. 3. Temporal relationship of poly(ICLC)-induced interferon response in mice to time of irradiation for maximum radioprotection, (A) 48 hr, (B) 72 hr, (C) 24 and 48 hr, and (D) 24, 48 and 72 hr. ↑, poly(ICLC); ▲, 3.0 mg/kg; ▼, 1.0 mg/kg; ■, 0.3 mg/kg.

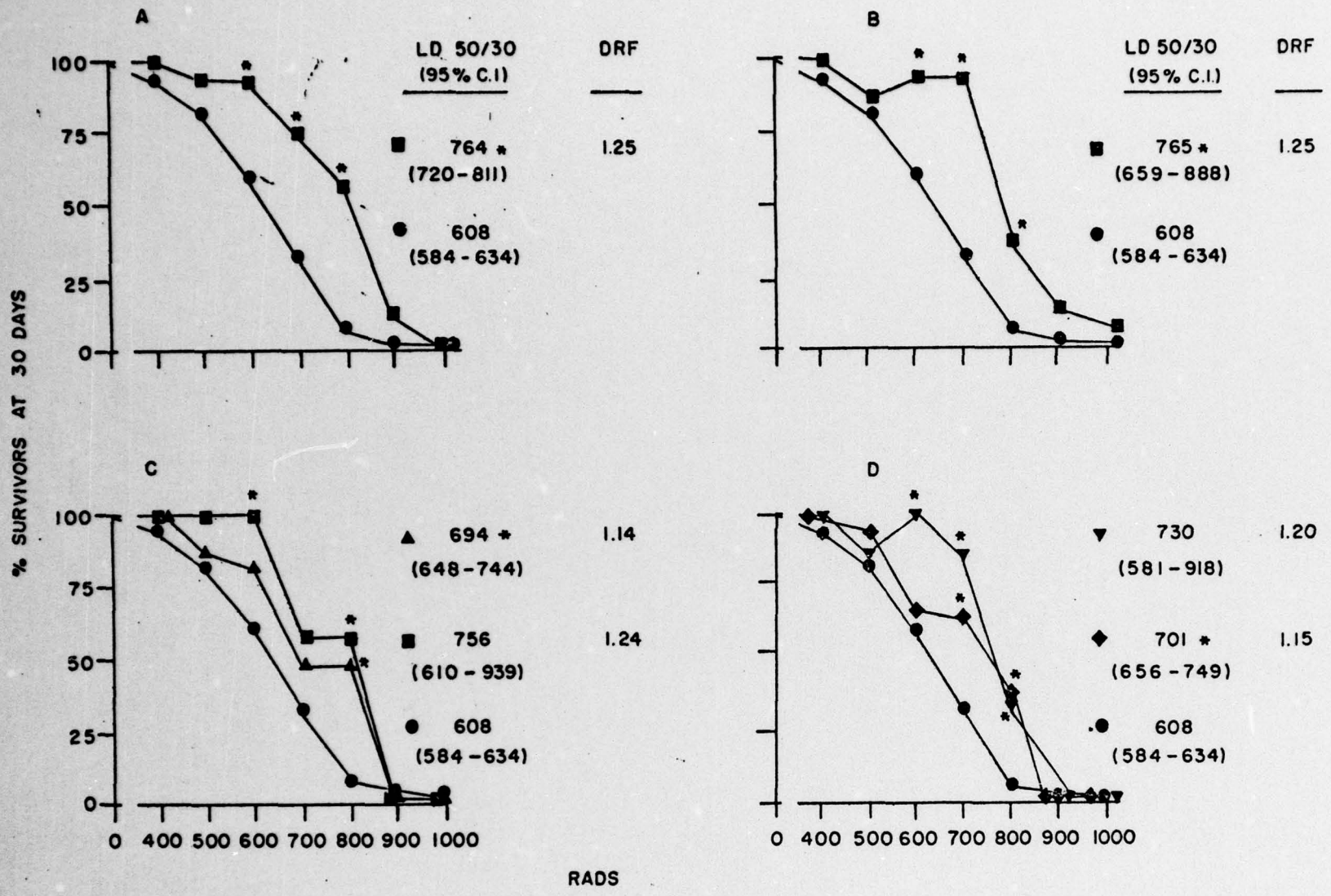
A
B
C
D
LD 50/30 (95% C.I.)
DRF
764 * (720-811) 1.25
608 (584-634)
765 * (659-888) 1.25
608 (584-634)
694 * (648-744) 1.14
756 (610-939) 1.24
608 (584-634)
730 (581-918) 1.20
701 * (656-749) 1.15
608 (584-634)
% SURVIVORS AT 30 DAYS
100
75
50
25
0
0 400 500 600 700 800 900 1000
RADS

Fig. 1

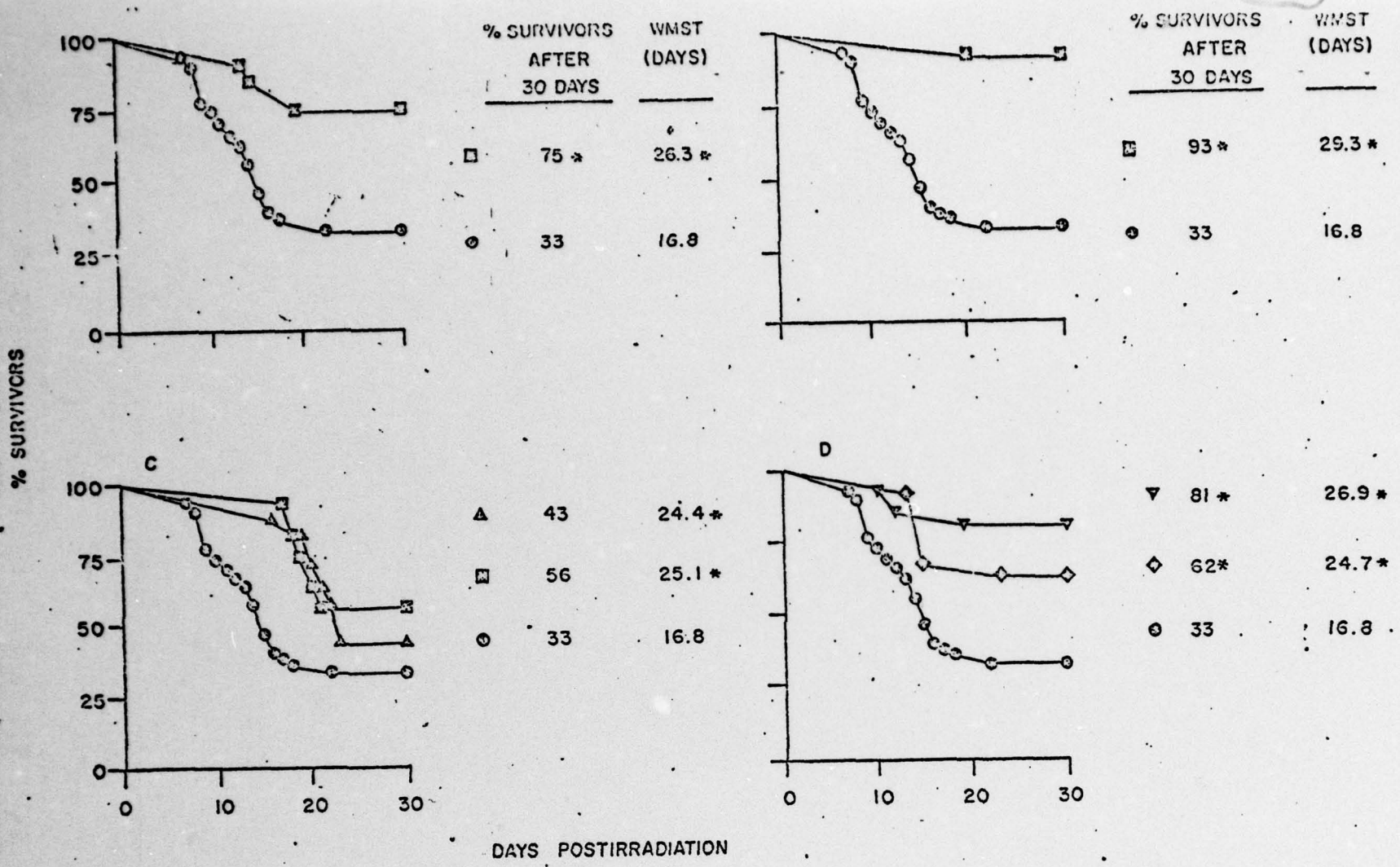
% SURVIVORS
100
75
50
25
0
0
10
20
30
DAYS POSTIRRADIATION
C
D
% SURVIVORS AFTER 30 DAYS
WMST (DAYS)
75 *
26.3 *
33
16.8
93 *
29.3 *
33
16.8
43
24.4 *
56
25.1 *
33
16.8
81 *
26.9 *
62*
24.7 *
33
16.8

Fig. 2

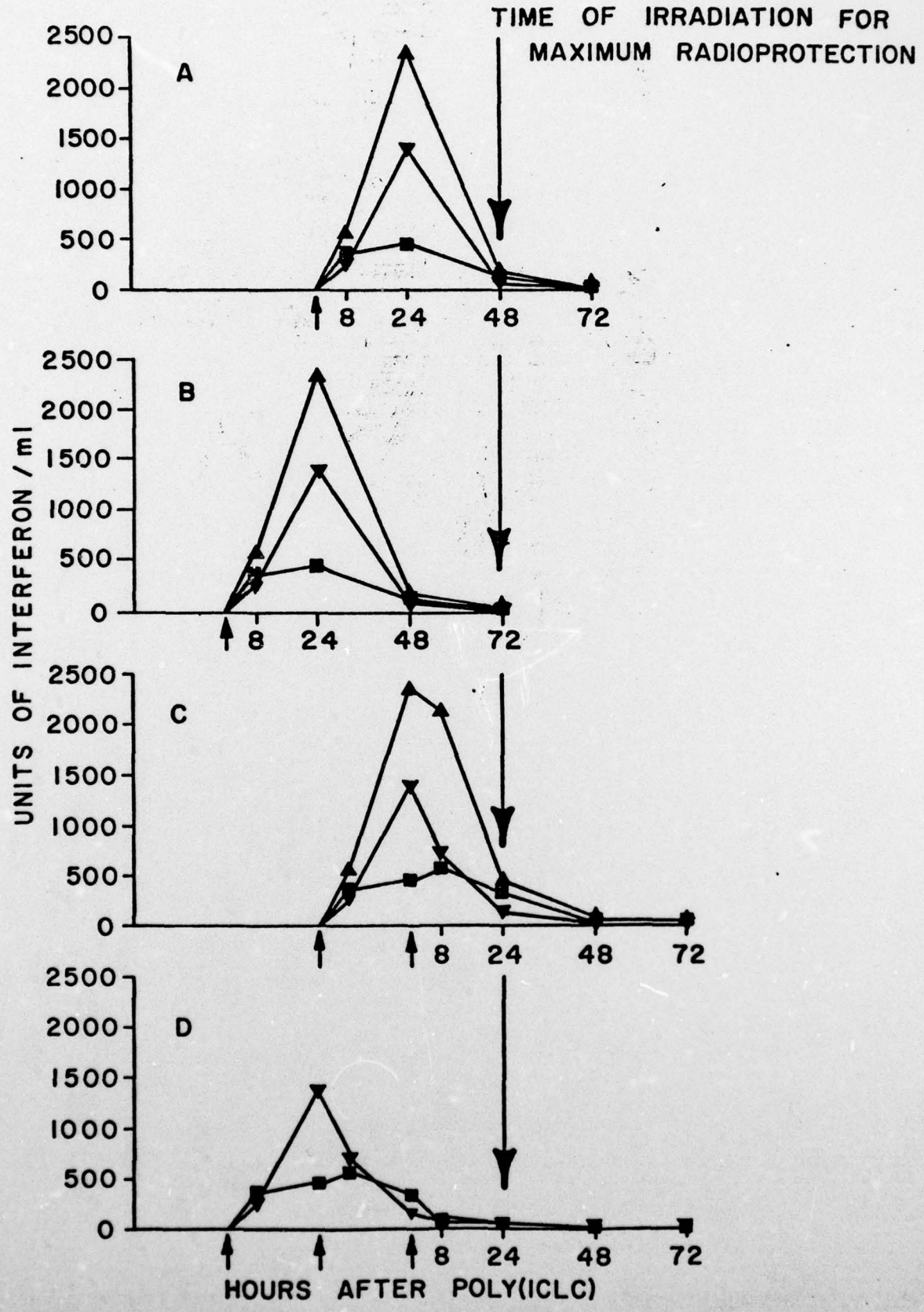
TIME OF IRRADIATION FOR MAXIMUM RADIOPROTECTION
A
B
C
D
2500
2000
1500
1000
500
0
8
24
48
72
UNITS OF INTERFERON / ml
HOURS AFTER POLY(ICLC)

Fig. 3